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L2: Entry 6 of 18

File: USPT

Feb 22, 2000

DOCUMENT-IDENTIFIER: US 6028243 A

TITLE: Mice and cells with a homozygous disruption in the RNase L gene and methods therefore

DEPR:

To precisely map the functional domains in RNase L, nested deletions of both termini of human RNase L cDNA were expressed as N-terminal glutathione S-transferase (GST) fusion proteins from vector pGEX-4T-3 (Pharmacia) in *E. coli*. The human RNase L cDNA was subcloned downstream of the coding sequence for GST in expression vector pGEX-4T-3 (Pharmacia). The nucleotide sequence of human RNase L cDNA, SEQ. ID. NO. 1, and the predicted amino acid sequence, SEQ. ID. NO. 2 of the human RNase L enzyme are shown in Table 1. The deletion mutants of RNase L, which are depicted in FIG. 2, were constructed by PCR and restriction enzyme cleavages. All mutants were confirmed by DNA sequence analysis. Induction in *E. coli* was at 30.degree. C. with 0.1 mM IPTG for 5 h. To purify the fusion proteins, glutathione sepharose 4B (100 ml of a 50% slurry) was added to extract the protein from 50 ml of culture medium at room temperature for 30 min. After washing the protein-bead complexes three times with buffer, the fusion proteins were eluted with 20 mM of glutathione in 50 mM Tris-HCl, pH 8.0. Expression and purity of the protein preparations was determined by SDS/PAGE and coomassie blue staining and by probing Western blots with a monoclonal antibody to RNase L.

DEPR:

2-5A dependent binding of the mutant RNase L fusion proteins to native human RNase L (not a fusion protein) expressed in insect cells in the presence and absence of 2-5A was determined after immobilization on glutathione sepharose. This assay was performed by incubating 50 .mu.g of extracts containing wild type or mutants of RNase L fused to GST with recombinant human RNase L (25 .mu.g) from insect cells in the presence and absence of 0.8 .mu.M pA(2'p5'A).sub.3 on ice for 2 h. Subsequently, glutathione sepharose was added and the mixture was incubated with shaking at room temperature for 20 min. Analysis of the bound protein was by SDS/PAGE and western blot analysis probed with antibody to RNase L using the enhanced chemiluminescence (ECL) method (Amersham). The results are shown in FIG. 3. In FIG. 3, lanes 1 and 2 contain 1 .mu.g and 0.15 .mu.g of insect cell extract containing human recombinant RNase L. RNase L is indicated by the arrows and by the circle in panel B, lane 9.

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L4: Entry 1 of 2

File: USPT

Nov 7, 2000

DOCUMENT-IDENTIFIER: US 6143875 A

TITLE: Antibody to DNase involved in apoptosis

DEPR:

Said DNase .gamma. is useful as one of the tools for elucidating, at a molecular level, apoptosis which occurs in mammals (e.g., cow, horse, mouse, rat, guinea pig and rabbit) inclusive of human, as well as for the development of diagnostic using an antibody of DNase .gamma., apoptosis-controlling pharmaceutical products containing inhibitor or activating agent of DNase .gamma.; evaluation of apoptosis; and as a basis for establishing apoptotic gene therapy of cancer and autoimmune diseases.

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USPT	s adj 1 adj nuclease	27	<u>L4</u>
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L2: Entry 5 of 18

File: USPT

Aug 29, 2000

DOCUMENT-IDENTIFIER: US 6110968 A

TITLE: Methods for treatment predicated on the presence of advanced glycosylation endproducts in tobacco and its combustion byproducts

DRPR:

FIG. 6 is a further series of graphs showing that glycotoxins are found in cigarette smoke (A). Glycotoxins from mainstream smoke can covalently crosslink RNase A proteins (and this cross-linking is inhibited by aminoguanidine). RNase A was exposed to mainstream cigarette smoke for 0, 5, 8, 24, 48 and 72 hours. To assess the formation of dimers, the samples were subjected to SDS-PAGE under reducing conditions, followed by transfer to nitrocellulose and western blotting with a rabbit anti-RNase A antibody conjugated to HRP. Circles=RNase A alone; Squares=RNase A after incubation with cigarette smoke; Triangles=RNase A after incubation with cigarette smoke and 5 mM aminoguanidine; Diamonds=RNase A after incubation with cigarette smoke and 50 mM aminoguanidine. (B) Glycotoxins have autofluorescence. RNase A was exposed to mainstream cigarette smoke for 0, 5, 8, 24, 48 and 72 hours and then assayed for glycotxin-specific fluorescence by measuring emission at 440 nm upon excitation at 370 nm. Circles=RNase A alone; Squares=RNase A after incubation with cigarette smoke; Triangles=RNase A after incubation with cigarette smoke and 5 mM aminoguanidine; Diamonds=RNase A after incubation with cigarette smoke and 50 mM aminoguanidine.

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L2: Entry 16 of 18

File: USPT

Mar 2, 1993

DOCUMENT-IDENTIFIER: US 5190864 A

TITLE: Enzyme amplification by using free enzyme to release enzyme from an immobilized enzyme material

DEPR:

4. A monoclonal antibody is obtained by immunizing mice with bovine RNase following a standard protocol, (Goding, J. W., Monoclonal Antibodies: Principles and Practice, Academic Press, N.Y., 1983), screening for and cloning hybridoma cells that secrete an antibody that both inhibits RNase enzymatic activity and binds to RNase-agarose, giving anti-RNase antibody.

DEPR:

6. SPDP RNase is reduced with one equivalent of dithiothreitol, forming sulfhydryl RNase that is complexed with excess anti-RNase antibody, reacted with maleimido-poly C-Immobilon Membrane, and washed extensively at low pH in the presence of guanidine-HCl to remove antibody, followed by washing with phosphate buffer at pH 7 and then HEPES buffer at pH 7, forming RNase-poly C-Immobilon Membrane.

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(FILE 'HOME' ENTERED AT 08:50:09 ON 30 OCT 2001)

FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE' ENTERED AT 08:50:21 ON 30 OCT 2001

L1 4 S ANTI(W)RIBONUCLEASE(W)ANTIBOD?
L2 11 S ANTI(W)RNASE(W)ANTIBOD?
L3 8 DUPLICATE REMOVE L2 (3 DUPLICATES REMOVED)

=> s anti near0 ribonuclease near0 s1

L4 0 ANTI NEAR0 RIBONUCLEASE NEAR0 S1

=> s l2 (p)micrococcal

L5 0 L2 (P) MICROCOCCAL

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USPT	13 same (advantag\$ or useful\$)	2	<u>L4</u>
USPT	DNase near0 antibod\$	8	<u>L3</u>
USPT	RNase near0 antibod\$	18	<u>L2</u>
USPT	(nuclease near0 inhibit\$) same antibod\$	4	<u>L1</u>

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USPT	13 same (advantag\$ or useful\$)	2	<u>L4</u>
USPT	DNase near0 antibod\$	8	<u>L3</u>
USPT	RNase near0 antibod\$	18	<u>L2</u>
USPT	(nuclease near0 inhibit\$) same antibod\$	4	<u>L1</u>